American Journal of Hematology 57:187–192 (1998)

Content of Reduced Glutathione and Consequences in Recipients of Glucose-6-Phosphate Dehydrogenase Deficient Red Blood Cells

Ching-Shan Huang,[1]* Yung-Chan Sung,[2] May-Jen Huang,[1] Chung-Shi Yang,[4] Wei-Shang Shei,[1] and Tang K. Tang[3]

[1]Department of Laboratory Medicine, Cathay General Hospital, Taipei, Taiwan, Republic of China
[2]Department of Internal Medicine, Cathay General Hospital, Taipei, Taiwan, Republic of China
[3]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, Republic of China
[4]Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan, Republic of China

The red blood cell glucose-6-phosphate dehydrogenase (G6PD) activity of every donor was examined with automatic enzyme-coupled method. The technique of molecular biology was applied to determine the DNA mutations for the 97 donors with undetectable G6PD activity. The concentration of reduced glutathione (GSH) in the stored RBC of the 97 G6PD-deficient donors and 124 normal donors was determined with the technique of high performance liquid chromatography. Routine blood counts, bilirubin and haptoglobin levels were used to evaluate posttransfusional hemolysis for the 48 adult patients transfused with 1 U G6PD deficient and 1 U normal RBC. Most (88, 90.7%) of the 97 donors were confirmed to be G6PD deficient at the DNA level. At each age interval of storage, the GSH concentration of G6PD-deficient RBC was significantly different from that of normal RBC. The total average value of GSH (μmol/gHb) was 2.52 ± 0.95 (mean ± 1 standard deviation) vs. 3.74 ± 1.43 (*P* < 0.001). Hemoglobin, hematocrit, bilirubin, and haptoglobin levels in the patients receiving G6PD-deficient RBC were not statistically different from those in the recipients of normal RBC; even though the age of stored blood was 26–35 days. Within the same group of patients, the results of bilirubin and haptoglobin were not significantly changed before and after transfusion. The results of this study show that the GSH concentration in the stored blood of G6PD deficient donors was 67% of that in the normal donors. However, hemolysis does not occur in adult patients transfused with 1 U G6PD-deficient RBC. It seems unnecessary to screen G6PD activity for donors of adult recipients in Taiwan. Am. J. Hematol. 57:187–192, 1998.

Key words: G6PD deficiency; reduced glutathione; posttransfusional hemolysis reaction

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common one of all clinically significant enzyme defects. Since G6PD deficiency is definitely associated with hemolysis [1], studies concerning the mechanism of hemolytic reaction including that occurring in blood transfusion are important topics for G6PD deficiency. Exchange transfusion of G6PD-deficient blood has been found to cause hemolysis in infants [2,3] and exaggeration of neonatal hyperbilirubinemia [4]. It has been noted that posttransfusion viability of autologous G6PD-deficient A$^-$ blood was inferior to that of normal cells [5]. A minor hemolytic reaction as slight increases in bilirubin and lactate dehydrogenase level was found in adults receiving a unit of severely G6PD-deficient blood [6,7]. However, the mechanism of storage change in G6PD-deficient blood during conventional blood banking has never been studied.

The prevalence of G6PD deficiency in male Taiwanese is approximately 3.0% [8–10]. We and others have shown that at least 12 different types of G6PD mutation

Contract grant sponsor: National Science Council, R.O.C.; Contract grant number: NSC 84-2331-B-281-002.

*Correspondence to: Professor Ching-Shan Huang, Department of Laboratory Medicine, Cathay General Hospital, 280 Sec 4 Jen-Ai Road, Taipei, Taiwan, Republic of China.

Received for publication 9 May 1997; Accepted 27 August 1997

TABLE I. Primers, Restriction Enzymes, and the Results for the Three New G6PD Mutations*

Position (cDNA)	Primers	Sequence	Restriction enzyme	Results (bp)
517 [15]	517F:	5′GCGTCTGAATGATGCAGCTCTGAT3′	Xho I	Nr 134
T → C	517R:	5′GAGCTCTGCAGGTCCCTCTCGA3′		Mu 114 + 20
519 [18]	519F:	Same as for 517F	Sty I	Nr 300
C → G	519R:	5′GGCCAGGTGAGGCTCCTGAGTA3′		Mu 189 + 111
871 [10]	871F:	5′TGAGGGCTGCACATCTGTGG3′	Bgl II	Nr 118
G → A	871R:	5′GCACCTCTGAGATGCATTTCAAGA3′		Mu 94 + 24

*′–′: mutagenesis site; bp: base pair (size of PCR product); Nr: normal digestion result; Mu: mutant digestion result.

at the DNA level are responsible for G6PD deficiency in Taiwan [9–15]. Since most of the mutations in Taiwanese are severely deficient WHO-class 2 variants, it seems to be necessary to study the circumstance of transfusion from the donors with G6PD deficiency. In this report, we investigated the reduced glutathione (GSH) concentration of 97 G6PD-deficient stored blood and the consequences of 48 adult recipients transfused with that blood. To our knowledge no such large body of data has been reported previously.

MATERIALS AND METHODS

Donation Blood

All the donated blood was obtained from the Taipei Blood Donation Center. Blood donations of 250 ml (1 U, regulation set by the Taiwanese Department of Health) were collected in a standard commercial plastic bag with CPDA-1 as anticoagulant, then made to be packed RBC (180–200 ml) and stored at 2–6° in a blood bank for up to 35 days.

Determination of G6PD Activity and Mutation Types

The quantitation of G6PD activity for every donor's blood was measured by the automated enzyme-coupled method as described [16]. Any subjects with G6PD activity below 4.0 IU/gHb were defined as having G6PD deficiency. For the research of severely G6PD-deficient blood, 97 donors with undetectable G6PD activity (0 IU/gHb) were selected as the study group. The G6PD mutations were identified at the DNA level on the thermal cycler (Perkin Elmer Cetus, Norwalk, CT) by using the nine primers as described [10] and three additional primers as listed in Table I. The restriction enzymes were obtained from Biolabs Co. (Beverly, MA).

Determination of GSH

One milliliter of blood was lysed by ultrasonic vibration (full power for 5 min on Heat System XL 2020 Sonicator, Farmingdale, NY), then deproteinized with 3 ml of absolute methyl alcohol (obtained from JT Baker Co., Phillipsburg, NJ). After centrifugation (13,000*g* for 10 min), GSH of the supernatant was determined by the technique of high performance liquid chromatography (Hewlett-Parckard 1050 series, Waldbronn, Germany) as described [17]. The concentration of GSH in each specimen was expressed as μmol/gHb. The stored blood of 124 subjects with normal G6PD activity was chosen as the control.

Patients

All the recipients were adults. They did not have oxidative stress, did not have irregular antibodies, had normal liver function tests, normal bilirubin (0.2–1.2 mg/dl) (performed on Hitachi 747 analyzer, Tokyo, Japan) and haptoglobin (30–140 mg/dl) (performed on Beckman Array Protein System, Brea, CA) before transfusion, were not treated with oxidative medications liable to induce hemolysis in G6PD deficiency [1], and no more blood was transfused during the study period. In order to collect enough numbers (N = 16 for each group) for study, three groups of patients were chosen as the study subjects. There were patients with anemia of chronic disease, gastrointestinal tract bleeding, and blood loss of 100–250 ml during surgery, respectively. Each of the 48 study recipients was transfused with 1 U of G6PD-deficient packed RBC and 1 U age-matched normal packed RBC. The reason for this combination is that the probability that both units of the donated blood being G6PD deficient is very low, since the prevalence of G6PD deficiency in Taiwan is ≦3%. Each of the 48 control recipients was transfused with 2 U normal packed RBC. The time from phlebotomy to transfusion of all the units was divided into two groups, 11–20 days and 26–35 days. Tests of complete blood count (performed on Sysmex NE-8000, Kyoto, Japan), bilirubin, and haptoglobin were done for all recipients 24, 48, and 72 hr after transfusion.

Statistical Analysis

Linear regression, two-tailed Student's *t*-test and paired *t*-test as appropriate were used to analyze the data. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

The mutation types of the 97 donors with undetectable G6PD activity were analyzed as those listed in Table II.

TABLE II. G6PD Mutations of the 97 Donors With Undetectable G6PD Activity

Variant	Nucleotide substitution	N	%
Taiwan-Hakka	1,376	44	45.4
Kaiping	1,388	11	11.3
Taipei	493	7	7.2
Gaohe	95	6	6.2
Chinese-5	1,024	6	6.2
Chinese-4	392	3	3.1
Miaoli	519	3	3.1
Viangchan	871	3	3.1
Coimbra	592	2	2.1
Mahidol	487	1	1.0
Nankang	517	1	1.0
Union	1,360	1	1.0
	Unknown	9	9.3
	Total	97	100.0

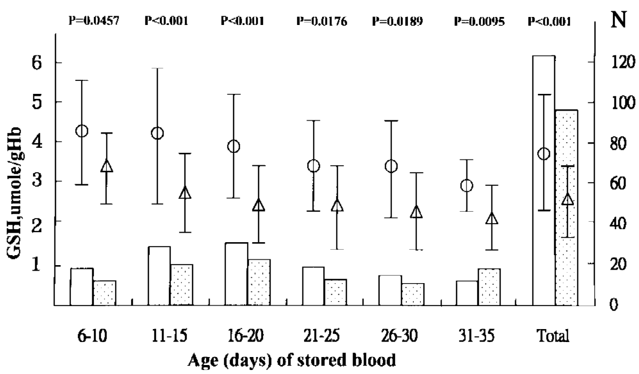

Fig. 1. The comparisons of GSH concentration between G6PD deficient subjects (△) and control subjects (○). (The heights of bars represent numbers of subjects).

Figure 1 shows that, in each age interval of stored blood, the GSH concentration (μmol/gHb) of G6PD-deficient subjects was significantly different from that of the control subjects. Mean ± 1 SD: 3.35 ± 0.86 vs. 4.25 ± 1.30 at 6–10 days, 2.69 ± 0.95 vs. 4.20 ± 1.73 at 11–15 days, 2.46 ± 0.99 vs. 3.86 ± 1.25 at 16–20 days, 2.34 ± 1.12 vs. 3.43 ± 1.23 at 21–25 days, 2.21 ± 0.92 vs. 3.35 ± 1.25 at 26–30 days, 2.11 ± 0.84 vs. 2.95 ± 0.76 at 31–35 days of storage, and 2.52 ± 0.95 vs. 3.74 ± 1.43 in total. The results of linear regression analysis for the relationship between GSH concentration (Y) and age (day, X) of stored blood were $Y = -0.033 \times +3.214$, $r = 0.278$ ($N = 97$, $P = 0.0080$) for G6PD deficient subjects and $Y = -0.055 \times +4.740$, $r = -0.274$ ($N = 124$, $P = 0.0037$) for control subjects, respectively.

The data listed in Table III indicate that there was no difference in the severity of the anemia between the study group and control group in each of the three kinds of patients. Table III also shows that the effectiveness in correcting the anemia by transfusing G6PD-deficient RBC was equal to that of normal RBC even though the age of blood was 26–35 days. As shown in Table IV, the

TABLE III-a. The Comparisons of Hemoglobin (Hb, g/dl) and Hematocrit (Ht, %) Between Recipients of G6PD-Deficient (G6PDd) RBC and Recipients of Normal RBC

	Before transfusion		72 hr after transfusion	
	G6PDd[a]	normal[b]	G6PDd[a]	normal[b]
Age of blood: 11–20 days				
Anemia of chronic disease				
Hb				
Range	7.6–10.0	6.7–9.8	10.6–13.6	9.5–12.9
$\bar{x}$ ± SD	8.8 ± 0.8	8.7 ± 0.9	11.2 ± 1.2	11.0 ± 1.3
Ht				
Range	22.7–30.5	23.6–29.7	30.4–39.0	29.5–38.5
$\bar{x}$ ± SD	27.2 ± 2.4	26.7 ± 2.7	33.5 ± 3.4	32.7 ± 3.6
Gastrointestinal tract bleeding				
Hb				
Range	7.9–9.4	6.7–10.4	9.1–11.1	9.9–12.0
$\bar{x}$ ± SD	8.2 ± 0.9	8.3 ± 1.1	10.0 ± 0.8	10.3 ± 1.0
Ht				
Range	21.4–30.5	20.7–31.9	26.2–32.5	28.5–35.5
$\bar{x}$ ± SD	24.9 ± 3.0	25.4 ± 4.1	29.3 ± 2.4	30.1 ± 3.0
Blood loss during surgery				
Hb				
Range	7.6–14.0	9.2–14.3	8.8–14.2	9.8–14.5
$\bar{x}$ ± SD	10.9 ± 2.5	11.8 ± 2.4	12.3 ± 1.4	12.6 ± 1.5
Ht				
Range	21.9–40.4	28.7–43.0	32.2–43.1	35.2–44.0
$\bar{x}$ ± SD	32.8 ± 7.4	35.8 ± 7.5	35.5 ± 5.6	37.4 ± 6.0

[a,b]: The number of recipients was 10, respectively. The concentrations of Hb and Ht were not statistically different between the two groups of recipients.

TABLE III-b. The Comparisons of Hemoglobin (Hb, g/dl) and Hematocrit (Ht, %) Between Recipients of G6PD-Deficient (G6PDd) RBC and Recipients of Normal RBC

	Before transfusion		72 hr after transfusion	
	G6PDd[a]	normal[b]	G6PDd[a]	normal[b]
Age of blood: 26–35 days				
Anemia of chronic disease				
Hb				
Range	7.5–10.5	7.6–10.3	9.9–12.6	9.7–12.8
$\bar{x}$ ± SD	9.0 ± 1.0	8.7 ± 0.9	10.8 ± 1.1	10.9 ± 1.2
Ht				
Range	24.6–31.9	22.8–31.3	28.8–38.8	29.3–37.9
$\bar{x}$ ± SD	27.6 ± 2.6	26.5 ± 2.8	32.8 ± 3.3	32.6 ± 3.5
Gastrointestinal tract bleeding				
Hb				
Range	7.1–11.2	6.8–10.8	9.9–12.4	9.5–12.2
$\bar{x}$ ± SD	8.7 ± 1.1	8.5 ± 1.0	10.6 ± 1.2	10.2 ± 1.3
Ht				
Range	21.3–32.5	20.9–31.5	27.6–38.7	28.0–37.5
$\bar{x}$ ± SD	27.1 ± 2.6	26.4 ± 3.0	32.0 ± 2.9	31.1 ± 2.8
Blood loss during surgery				
Hb				
Range	9.1–14.4	9.4–13.3	9.7–15.2	9.9–15.0
$\bar{x}$ ± SD	11.8 ± 2.6	11.6 ± 2.5	12.5 ± 1.7	12.0 ± 1.8
Ht				
Range	28.4–42.4	27.5–39.5	29.0–46.8	31.8–43.4
$\bar{x}$ ± SD	35.5 ± 6.7	34.9 ± 5.8	38.0 ± 6.9	36.2 ± 6.1

[a,b]: The number of recipients was six, respectively. The concentrations of Hb and Ht were not statistically different between the two groups of recipients.

TABLE IV-a. The Comparisons of Bilirubin (mg/dl) and Haptoglobin (mg/dl) Between Recipients of G6PD-Deficient (G6PDd) RBC and Recipients of Normal RBC

	Before transfusion		After transfusion 24 hr		48 hr		72 hr	
	G6PDd[a]	normal[b]	G6PDd[a]	normal[b]	G6PDd[a]	normal[b]	G6PDd[a]	normal[b]
Age of blood: 11–20 days								
Anemia of chronic disease								
Bilirubin								
Range	0.2–0.5	0.2–0.6	0.2–0.6	0.2–0.6	0.3–0.6	0.3–0.6	0.2–0.5	0.2–0.6
$\bar{x} \pm SD$	0.31 ± 0.10	0.30 ± 0.09	0.36 ± 0.14	0.35 ± 0.15	0.37 ± 0.13	0.37 ± 0.15	0.32 ± 0.12	0.33 ± 0.11
Haptoglobin								
Range	35–132	28–135	40–124	33–120	36–138	41–134	33–135	42–138
$\bar{x} \pm SD$	81 ± 29	75 ± 31	77 ± 27	70 ± 28	82 ± 30	80 ± 28	80 ± 26	76 ± 25
Gastrointestinal tract bleeding								
Bilirubin								
Range	0.2–0.9	0.2–0.9	0.2–0.9	0.2–0.8	0.2–0.9	0.2–0.9	0.2–0.7	0.2–0.8
$\bar{x} \pm SD$	0.50 ± 0.29	0.43 ± 0.21	0.44 ± 0.29	0.45 ± 0.20	0.48 ± 0.25	0.49 ± 0.23	0.44 ± 0.25	0.43 ± 0.21
Haptoglobin								
Range	35–132	33–131	33–124	29–135	41–130	38–138	37–138	35–133
$\bar{x} \pm SD$	88 ± 28	90 ± 29	80 ± 25	84 ± 26	82 ± 24	80 ± 23	85 ± 27	87 ± 26
Blood loss during surgery								
Bilirubin								
Range	0.3–0.7	0.3–0.7	0.3–0.8	0.3–0.8	0.3–0.8	0.3–0.8	0.3–0.9	0.3–0.8
$\bar{x} \pm SD$	0.41 ± 0.14	0.39 ± 0.15	0.52 ± 0.17	0.45 ± 0.16	0.50 ± 0.23	0.45 ± 0.21	0.50 ± 0.22	0.42 ± 0.19
Haptoglobin								
Range	39–110	41–94	35–105	36–107	40–112	36–102	33–108	35–100
$\bar{x} \pm SD$	76 ± 24	70 ± 22	68 ± 23	66 ± 24	74 ± 26	64 ± 23	70 ± 27	71 ± 26

[a,b]: The number of recipients was 10, respectively. The concentrations of bilirubin and haptoglobin were not statistically different between the two groups of recipients.

TABLE IV-b. The Comparisons of Bilirubin (mg/dl) and Haptoglobin (mg/dl) Between Recipients of G6PD-Deficient (G6PDd) RBC and Recipients of Normal RBC

	Before transfusion		After transfusion 24 hr		48 hr		72 hr	
	G6PDd[a]	normal[b]	G6PDd[a]	normal[b]	G6PDd[a]	normal[b]	G6PDd[a]	normal[b]
Age of blood: 26–35 days								
Anemia of chronic disease								
Bilirubin								
Range	0.2–0.7	0.2–0.6	0.2–0.8	0.2–0.7	0.2–0.8	0.2–0.7	0.2–0.7	0.2–0.8
$\bar{x} \pm SD$	0.30 ± 0.11	0.31 ± 0.12	0.35 ± 0.13	0.33 ± 0.15	0.34 ± 0.16	0.35 ± 0.17	0.34 ± 0.14	0.34 ± 0.15
Haptoglobin								
Range	38–128	34–131	34–121	32–123	29–124	38–130	39–129	35–136
$\bar{x} \pm SD$	87 ± 32	80 ± 30	78 ± 30	72 ± 28	75 ± 28	77 ± 31	83 ± 30	80 ± 38
Gastrointestinal tract bleeding								
Bilirubin								
Range	0.2–1.0	0.2–0.8	0.2–0.7	0.2–0.8	0.3–0.8	0.2–0.8	0.2–0.7	0.2–0.7
$\bar{x} \pm SD$	0.50 ± 0.33	0.43 ± 0.21	0.45 ± 0.21	0.46 ± 0.28	0.47 ± 0.21	0.45 ± 0.19	0.44 ± 0.15	0.45 ± 0.18
Haptoglobin								
Range	30–130	42–135	27–127	40–131	36–130	41–122	40–127	51–132
$\bar{x} \pm SD$	77 ± 29	85 ± 30	79 ± 28	79 ± 31	83 ± 30	78 ± 28	80 ± 33	83 ± 32
Blood loss during surgery								
Bilirubin								
Range	0.2–0.6	0.2–0.7	0.2–1.1	0.2–0.8	0.2–0.9	0.2–0.8	0.2–0.8	0.2–0.7
$\bar{x} \pm SD$	0.36 ± 0.15	0.34 ± 0.16	0.44 ± 0.20	0.35 ± 0.17	0.40 ± 0.19	0.36 ± 0.12	0.38 ± 0.18	0.37 ± 0.16
Haptoglobin								
Range	41–128	36–111	35–119	38–121	40–123	43–130	41–127	37–127
$\bar{x} \pm SD$	84 ± 28	75 ± 30	80 ± 25	73 ± 28	78 ± 23	75 ± 22	81 ± 23	84 ± 26

[a,b]: The number of recipients was six, respectively. The concentrations of bilirubin and haptoglobin were not statistically different between the two groups of recipients.

concentrations of bilirubin and haptoglobin in the recipients transfused with G6PD-deficient RBC of both 11–20 and 26–35 days old were not different from those in the recipients of age-matched normal RBC at pretransfusion, 24, 48, and 72 hr after transfusion. The results of the paired *t*-test in each kind of patients also showed that there was no significant difference in bilirubin and haptoglobin between 24, 48, or 72 hr after transfusion and pretransfusion.

DISCUSSION

We [9,10,14,15] and other scientists [11–13] have found that at least 12 different types of mutation are responsible for Taiwanese with G6PD deficiency. Those data also show that among the mutation types the nucleotide substitution (nt) of 1,376 predominates. In this report, we show the occurrence rates of the 12 types in the 97 donors with undetectable G6PD activity are similar to those we found in neonates [10], except that nt 517 [15] and nt 519 [18] are new findings. Our results also confirm that most (>90%) of the 97 donors are G6PD deficient at the DNA level. The nt 1,376 accounts for 45.4% of the 97 subjects, and is also the most popular variant.

G6PD is responsible for the generation of nicotinamide-adenine-dinucleotide phosphate (NADPH) in the hexose monophosphate pathway (HMP). The HMP is the only source of NADPH in RBC [1]. NADPH is a cofactor involved in maintaining glutathione in the reduced form (GSH), which is an important substance in the protection of RBC against oxidative damage [19]. Beutler et al [20] reported that content of GSH was lower in primaquine sensitive red cells than in normal cells. Al-Ali [21] has observed a decreased value of GSH in the subjects with G6PD deficiency when compared to normal. In this investigation, we also find that the GSH concentration in the stored blood of G6PD-deficient donors was statistically different from that of normal RBC. The former was 67% of the latter in average. Moreover, our results indicate that the GSH value slightly declines with the age of storage in both G6PD-deficient and normal RBC.

A decrease in the GSH level may influence the life span of stored RBC in some ethnics. Shalev et al. [6] have found that an immediate posttransfusional hemolysis reaction occurred in 6 of 10 recipients of G6PD-deficient RBC in Israeli subjects. Although they did not examine the GSH concentration for that blood, the authors postulate that the oxidation-mediated storage lesions that occurred in normal RBC are amplified in G6PD deficiency, further restricting their posttransfusional life-span, eventuating in posttransfusional hemolysis. In contrast, McCurdy and Morse [22] and Kuhn et al. [23] reported that the evaluation of the patients who received G6PD-A^- blood failed to reveal any hemolytic reaction.

We did not find any hemolysis cases in our study subjects, since the serum bilirubin and haptoglobin values in the recipients of G6PD-deficient RBC were not significantly changed after transfusion and were not different from those in the control subjects. The contradiction between our results and those from Shalev et al. [6] may be due to different types of G6PD mutation in the two groups studied. The most popular type of mutations in G6PD-Mediterranean is nt 563 with a silent mutation at nt 1,311 [24], while nt 1,376 predominates in Taiwan. Different types of G6PD mutation may lead to different biochemical and clinical characteristics. For instance, G6PD-Mediterranean owns a B-like electrophoretic mobility [24] and has been found to cause moderate hemolysis during the steady state [25], while nt 1,376 shows fast electrophoretic mobility [26] and is highly associated with neonatal jaundice in Taiwanese [10,13]. Therefore, different outcomes of blood storage may occur among different types of G6PD mutation. The decreases of GSH and antioxidant function in G6PD-Mediterranean stored blood may be more severe than those in G6PD-nt 1,376. Since posttransfusional hemolysis reaction is not observed in our patients transfused with G6PD-deficient RBC, it seems unnecessary to screen G6PD activity for blood donors of adult recipients in Taiwan.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Science Council, R.O.C. (NSC 84-2331-B-281-002)

REFERENCES

1. Beutler E: G6PD deficiency. Blood 84:3613–3636, 1994.
2. Mimouni F, Shohat S, Reisner SH: G6PD-deficient donor blood as a cause of hemolysis in two preterm infants. Isr J Med Sci 22:120–122, 1986.
3. Kumar P, Sarkar S, Narang A: Acute intravascular hemolysis following exchange transfusion with G6PD deficient blood. Eur J Pediatr 153:98–99, 1994.
4. Gulati S, Singh S, Narang A, Bhakoo ON: Exchange transfusion with G6PD deficient donor blood causes exaggeration of neonatal hyperbilirubinemia. Ind Pediatr 26:499–501, 1989.
5. Orlina AR, Josephson AM, McDonald BJ: The poststorage viability of glucose-6-phosphate dehydrogenase deficiency erythrocyte. J Lab Clin Med 75:934–936, 1970.
6. Shalev O, Manny N, Sharon R: Posttransfusional hemolysis in recipients of glucose-6-phosphate dehydrogenase deficient erythrocyte. Vox Sang 14:94–98, 1993.
7. Shalev O, Bogomolski-Yahalom V, Sharon R: Hemolysis following transfusion of erythrocytes from a donor with G6PD deficiency and beta-thalassemia minor. Isr J Med Sci 29:214–216, 1993.
8. Yu MW, Hsiao KJ, Wuu KD, Chen CJ: Association between glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice: Interaction with multiple risk factors. Int J Epidemiol 21:947–952, 1992.
9. Tang TK, Huang CS, Huang MJ, Tam KB, Yeh CH, Tang CJC:

Diverse point mutations result in glucose-6-phosphate dehydrogenase (G6PD) polymorphism in Taiwan. Blood 79:2135–2140, 1992.

10. Huang CS, Hung KL, Huang MJ, Li YC, Liu TH, Tang TK: Neonatal jaundice and molecular mutations in glucose-6-phosphate dehydrogenase deficient newborn infants. Am J Hematol 51:19–25, 1996.
11. Chang JG, Chiou SS, Pergn LI, Chen TC, Liu TC, Lee LS, Chen PH, Tang TK: Molecular characterization of G6PD deficiency by natural and amplification created restriction sites: Five mutations account for most G6PD deficiency cases in Taiwan. Blood 80:1079–1082, 1992.
12. Chiu DTY, Zuo L, Chao L, Chen E, Louie E, Lubin B, Liu TZ, Du CS: Molecular characterization of glucose-6-phosphate dehydrogenase (G6PD) deficiency in patients of Chinese descent and identification of new base substitutions in the human G6PD gene. Blood 81:2150–2154, 1993.
13. Lo YS, Lu CC, Chiou SS, Chen BH, Chang TT, Chang JG: Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in Chinese infants with or without severe neonatal hyperbilirubinemia. Br J Hematol 86:858–862, 1993.
14. Tang TK, Yeh CH, Huang CS, Huang MJ: Expression and biochemical characterization of human glucose-6-phosphate dehydrogenase in *Escherichia coli:* A system to analyze normal and mutant enzyme. Blood 83:1436–1441, 1994.
15. Chen HL, Huang MJ, Huang CS, Tang TK: G6PD Nankang (517 T → C; 173 Phe → Leu): A new G6PD variant identified in a Chinese associated with neonatal jaundice. Hum Hered 46:201–204, 1996.
16. Huang CS, Chen TH, Wei C, Chien TY, Jang JF: The clinical application of glucose-6-phosphate dehydrogenase quantitation test. J Formosan Med Assoc 81:938–944, 1982.
17. Yang CS, Chou ST, Liu L, Tsai PJ, Kuo JS: Effect of aging on human plasma glutathione concentrations as determined by high-performance liquid chromatography with fluorimetric detection. J Chromatogr 674:23–30, 1995.
18. Chen HL, Liu TH, Huang MJ, Huang CS, Tang TK: Two novel glucose-6 phosphate dehydrogenase deficiency mutations and association of such mutations with F8C/G6PD haplotype in Chinese. J Formosan Med Assoc 1997 (in press).
19. Wolle LC: Oxidative injuries to the red cell membrane during conventional blood preservation. Sem Hematol 26:307–312, 1989.
20. Beutler E, Dern RJ, Flanagan CL, Alving AS: The hemolytic effect of primaquine: VII. biochemical studies of drug-sensitive erythrocytes. J Lab Clin Med 45:286–295, 1955.
21. Al-Ali AK: Erythrocyte reduced glutathione level in sickle cell anemia and glucose-6-phosphate dehydrogenase deficient Saudi subjects. Ann Clin Biochem 31:296–297, 1994.
22. McMurdy PR, Morse EE: Glucose-6-phosphate dehydrogenase deficiency and blood transfusion. Vox Sang 28:230–237, 1975.
23. Kuhn VL, Lisboa V, de Cerqueira LP: Glucose-6-phosphate dehydrogenase in blood donors in a general hospital of Salvador, Bahia, Brazil. Rev Paul Med 101:175–177, 1983.
24. Kurdi-Haidar B, Mason PJ, Berrebi A, Ankra-Badu G, Al-Ali A, Oppenheim A, Luzzatto L: Origin and spread of the glucose-6-phosphate dehydrogenase variant (G6PD-Mediterranean) in the Middle East. Am J Hum Genet 47:1013–1019, 1990.
25. Ragusa R, Cataldo AD, Gangarossa S, Nigro LL, Schiliro G: Low grade hemolysis and assessment of iron status during the steady state in G6PD-deficient subjects. Acta Haematol 90:25–28, 1993.
26. Stevens DJ, Wanachiwanawin W, Mason PJ, Vulliamy TJ, Luzzatto L: G6PD Canton a common deficient variant in South East Asia caused by a 459 Arg → Leu mutation. Nucleic Acids Res 18:7190, 1990.